RESTRICTED CIRCULATION

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AGRICULTURAL MEMORANDUM NO 942

Newcastle disease - Part 1 - A Literature search for evidence of spread of disease by airborne virus.

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INTRODUCTION

On 1 September 1981 the Newcastle Disease (England and Wales) Order, 1971 was revoked. Newcastle disease (ND) is now controlled using the Fowl Pest (England and Wales) Order, 1936. As a result of this change of legislation the routine policy, under which poulty were vaccinated against the disease, was abolished. Consequentially, now that the protection afforded by the previous vaccination policy has worn off, poultry in England and Wales are unprotected against the disease.

When ND occurs in England or Wales it is the responsibility of the Ministry of Agriculture, Fisheries and Food (MAFF) to ensure that further spread of the disease is kept to a minimum. To achieve this it is necessary for MAFF to be aware of all of the possible ways by which disease can spread and, for any particular outbreak, to understand the relative importance of each of these routes. ND has been shown to be readily transmitted from flock to flock by movements of infected poultry, carriage of infected materials e.g. poultry crates, and by movement of people carrying virus on their hands or clothes, (Callander, 1958). In addition to these it has been shown that disease can result from airborne virus. The possibility of the spread of disease by contact can be determined by detailed investigations of the movements of poultry, people, materials etc. The likelihood of disease spread from one flock to another by airborne virus is more difficult to establish.

At the request of MAFF a joint project between the Central Veterinary Laboratory (MAFF), Weybridge and the Meteorological Office, Bracknell, has been commissioned to quantify the risk of the airborne spread of ND.

This article, after a general introduction to the disease, assembles the written evidence currently available to support the theory of the spread of the disease by airborne infected particles. As a result of this literature survey areas requiring further research are identified.

THE DISEASE

The following description of the disease is based upon articles written by Hanson (1972) and Brandley (1959).

ND is an infectious, highly contagious and destructive malady which attacks chiefly chickens and turkeys. Various other poultry and birds as well as certain mammals, including man, may also contract the disease. Over the years the disease has been given many names e.g. pseudo-fowl pest, pseudo-poultry plague, avian pest, avian distemper, Ranikhet and avian pneumoencephalitis. ND is caused by a virus which resembles the viruses of influenza and mumps in certain properties.

The virion or mature virus unit varies in size from 120 to 300.10^{-9}M but more usually 180.10^{-9}M (Waterson and Cruickshank (1963)). It consists of an envelope and an internal component. The envelope has a pattern of projections or spikes which are 80 Å long, Bong (1947). The internal component consists of a long much-coiled tube, with a diameter of 180 Å, Schafer and Rott (1959).

ND manifests itself in several different forms. Traditionally the names given to these forms have some from the workers who first detected them; Doyle, Beach, Beaudette and Hitchner.

a. Doyle's Form:

Doyle's form is an acute lethal infection of chickens of all ages and was first identified in 1926 near to Newcastle, England, Doyle (1927). A high mortality rate of 50-100% in adult birds has characterised this form of disease. Hemorrhagic lesions of the digestive tract are a prominent feature.

b. Beach's Form:

First recognised in 1942, Beach (1942) it is an acute and frequently lethal infection of chickens of all ages. Disease is characterised by lesions in the respiratory tract and nervous system. Hemorrhages are conspicuously absent from the digestive tract. It was initially called nervous respiratory disease or pneumoencephalitis.

c. Beaudette's Form:

This is an acute respiratory and sometime lethal nervous infection of young chickens. In older birds mortality is rare.

d. Hitchner's Form:

This form of ND is a mild or inapparent respiratory infection. Mortality is rare in birds of any age.

Currently the form of disease is classified according to its virulence and the organs principally affected. Three types of virus ranging from Velogenic (the most virulent) through mesogenic to lentogenic (the most avirulent) have been identified. The methods used to distinguish between the types is given by Gloster (1982a).

Examples of types of virus:

Velogenic - Herts '33/56, Eastwood '67, Essex '70, Milano, GB.
Mesogenic - Roakin, MK107
Lentogenic - B1, F, La Sota.

Having identified the type of virus the disease is further classified according to the organs principally affected e.g.

Pneumotropic - respiratory region.
Viscerotropic - gut region
Neurotropic - brain and nervous system.

Example of full classification: Essex '70 is defined as pneumotropic velogenic.

LITERATURE SURVEY

INTRODUCTION

Airborne spread of disease relies upon a complex chain of events. There are three main phases; firstly a period of virus excretion, secondly the virus has to be carried towards a susceptible host and thirdly this virus has to initiate infection. During each of these phases various factors are important and these are shown in Figure 1. Information on all of these factors is required if an accurate assessment of the potential for airborne disease spread is to be made.

The following literature survey provides information on some of these factors and identifies the areas where further research needs to be undertaken.

The literature has been divided into three main sections:

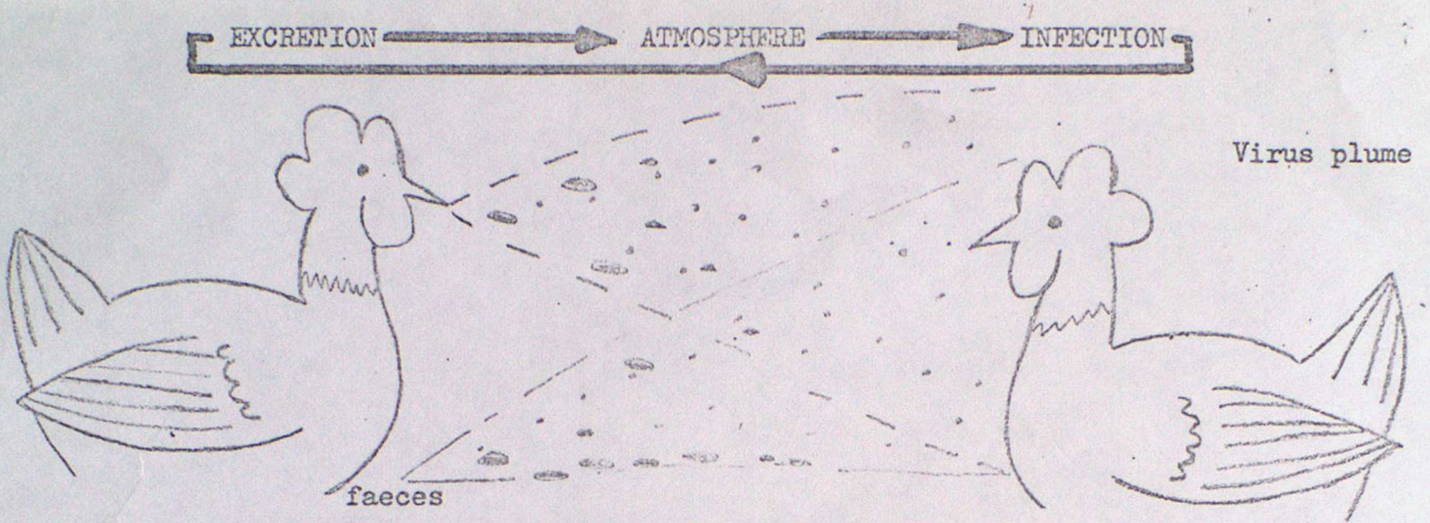
1. Airborne virus excretion and secondary airborne infection.
2. Virus survival
3. Airborne transmission of disease (field evidence).

Where a paper deals with one or more of the categories above it has been put into the most relevant section.

AIRBORNE VIRUS EXCRETION AND SECONDARY AIRBORNE INFECTION

Delay and others (1948)

Two air samples were taken from two separate poultry houses where ND had been present for several days. 540 and 1080 litres of air were drawn through allantoic fluids (fluid which comes from the allantoic-sac) harvested from normal 10-day old chick embryos. 13 out of 15,



Number of birds affected

Virus strain

Period of excretion (each bird and whole flock)

Age/size of birds

Route of excretion (Respiratory or faeces)

The effect of the environment on Virus excretion

Size of the particles containing Virus (large particles fall out of the atmosphere)

Turbulence

Virus inactivation (Temperature, Relative Humidity, Sunlight, Open air factor)

Number of susceptible birds

Condition of birds

Route of infection (Respiratory, occular etc)

Age of birds

Minimum dosage required to cause infection by various routes.

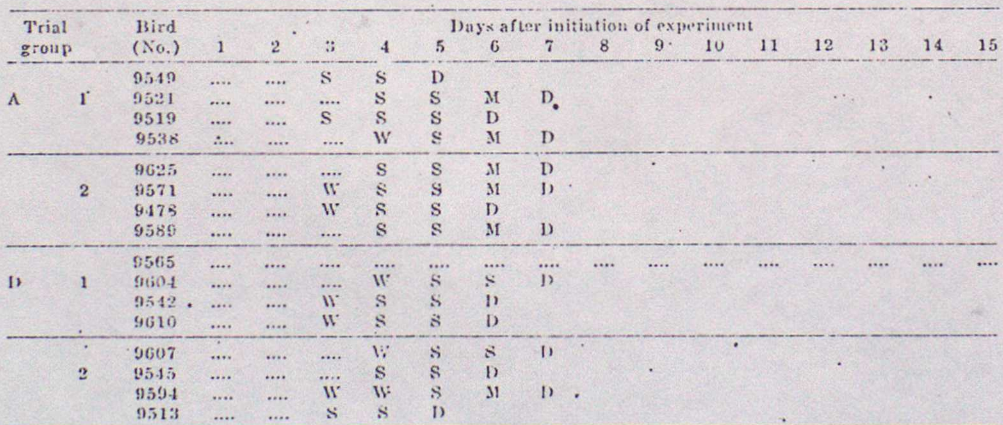
Figure 1. Factors which influence airborne disease spread

11 day old chick embroys injected with 0.2 ml samples of the allantoic fluid contaminated with air from the first poultry house, died. The mean time to death was 6.5 days post inoculation. Similar results were obtained with the samples taken from the second poultry house.

Sinha and others (1954)

Two separate groups of 4 birds, all 6 to 8 weeks old were infected with GB virus via the respiratory system. They were then housed in cages 1 ft away from two other groups of 4 birds. Table 1 shows the development of disease in the artificially infected birds and Table 2 the subsequent disease in the second group of birds.

Table 1 - Development of ND in chickens after exposure to artificial aerosols.



Trial	group	Bird	Days after initiation of experiment														
		(No.)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		9549	....	....	S	S	D										
A	1	9521	....	....	....	S	S	M	D								
		9519	....	....	S	S	S	D									
		9538	....	....	....	W	S	M	D								
		9625	....	....	....	S	S	M	D								
	2	9571	....	....	W	S	S	M	D								
		9478	....	....	W	S	S	D									
		9589	....	....	....	S	S	M	D								
		9565	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
B	1	9604	....	....	....	W	S	S	D								
		9542	....	....	W	S	S	D									
		9610	....	....	W	S	S	D									
		9607	....	....	....	W	S	S	D								
	2	9545	....	....	....	S	S	D									
		9594	....	....	W	W	S	M	D								
		9513	....	....	S	S	D										

Average time until signs appeared—4.1 days.

W = Weak; S = Signs; M = Moribund; D = Dead

Table 2 - Development of ND in chickens after exposure to natural aerosols (source of infection was air from cages of chickens developing first signs on Day 3 and dying by Day 7).

Trial group		Bird (No.)	Days after initiation of experiment														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B	1	9473	....	....	....	....	....	....	S	S	D						
		9479	....	....	....	....	....	....	S	S	S	D					
		9485	....	....	....	....	....	W	S	S	D						
		9488	....	....	....	....	....	....	....	S	S	M	D				
	2	9402	....	....	....	....	....	....	W	S	S	S	D				
		9493	....	....	....	....	....	....	W	S	S	S	D				
		9497	....	....	....	....	....	....	....	....	....	S	S	D			
		9499	....	....	....	....	....	....	....	....	....	....	S	S	S	D	
C	1	9469	....	....	....	....	....	....	....	....	....	....	S	S	D		
		9471	....	....	....	....	....	....	....	....	....	W	S	S	S	M	D
		9470	....	....	....	....	....	W	S	D							
		9482	...	....	....	....	....	....	....	S	S	S	(destroyed)				
	2	9487	....	....	....	....	....	....	....	....	S	S	S	S	D		
		9490	....	....	....	....	....	....	....	....	S	S	M	D			
		9511	....	....	....	....	....	....	....	S	S	S	D				
		9503	....	....	....	....	....	....	....	W	S	S	S	M	D		

Average time until signs appeared – 8.3 days (8.3-4.1-4.2).
Signs appeared 4.2 days after probable exposure.

ND virus was recovered from the air in the vicinity of the infected birds on the third, fourth, fifth and sixth day following initial exposure to virus. The authors estimated that there were from 1 to 10 virus particles present in an air sample of 0.5 litres.

Kohn (1955)

Experiments were performed to quantify the effect of infection of chicks by various routes; intranasal, respiratory, intratacheal, intramuscular and alimentary. Birds were infected with a highly pathogenic strain of ND virus (90% mortality in 7-14 day old birds). After infection each chick was housed in a separate wire cage with its own food and water.

Intranasal: The intranasal infective dose was found to be about 5000 ELD_{50} (Embryo lethal dosage, 50% mortality) for chickens aged 30-70 days. For 7 day old, parentially immune chicks the value was five fold higher. It was postulated that the dosage was high by the nasal route because the nasal mucous membrane was not very susceptible to virus.

Respiratory: Infection by the respiratory system for chickens aged 22 to 24 days was found to occur at 110 to 133 ELD_{50}. Results from a typical experiment are given in Tables 3 and 4.

Table 3 - Determination of ELD$_{50}$ of ND Virus strain HP by the respiratory (aerosol) route in 24-day-old chickens after a twenty-minute exposure.

						Results of infection					
						No. of chicks					
Exposure group	No. of chicks exposed	Ave. wt. (Gm.)	Ave. respiratory volume (ml./min.)	Exposed to e.l.d.$_{50}$ of NDV (Cv)	Total virus inhaled (e.l.d.$_{50}$)	Sick and paralyzed	Died of NDV without symptoms	HI titer† of symptomless survivors	Susceptible to challenge‡	Ratio infected to exposed	e.l.d.$_{50}$§
A	6	110	70	10	13	—	—	All negative	6	0/6	133
B	6	108	70	95	133	1	1	40	3	3/6	
C	6	128	85	1,250	2,100	5	—	160	—	6/6	
D	6	115	74	15,000	21,000	2	3	80	—	6/6	
Control	....	125	80	—	—	....	....	All negative	6	......	......

† Hemagglutination inhibition titer. ‡ 10^5 e.l.d.$_{50}$ intramuscularly, fourteen days after exposure. § Expressed in e.l.d.$_{50}$.

Table 4 - Determination of concentration of ND virus in the air during aerosol exposure of chickens

							Results of titration‡					
Exposure group	Total amount of NDV sprayed (e.l.d.$_{50}$)	Volume liquid (VF) in impingers (ml.)	Sampling time (min.)	Total air passed (liters)	Relative humidity (%)	Recovery† of NDV in the air (%)	Log dilution	Deaths of embryos	Log e.l.d.$_{50}$	e.l.d.$_{50}$ per ml.	e.l.d.$_{50}$ per liter air (Cv)	Cv. E §
B	6.1 x 10^5	22.2	6	54.0	75	4.4	0.5 1.0 1.5	4/4 4/4 3/4	1.66	46	94	95
		26.8	6	54.6	75	4.4	0.7 1.4 2.1	4/4 3/4 0/4	1.63	43	105	
C	5.0 x 10^6	24.7	5	46.0	70	6.2	1.0 1.7 2.4 3.1	4/4 3/4 3/3 0/4	2.63	427	1,140	1,250
D	6.0 x 10^7	24.1	5	46.0	79	6.4	2.0 3.0 4.0 5.0	4/4 4/4 1/4 0/4	3.75	5,620	1.47 x 10^4	15,000
		25.4	5	46.0	79	6.4	2.7 3.4 4.1 4.8	4/4 4/4 0/4 2/4	3.69	5,010	1.33 x 10^4	

† $\frac{\text{(Total amount of virus recovered from the air)}}{\text{Total amount of virus sprayed}} \times 100$ = percentage. ‡ Inoculum = 0.2 ml. § Concentration of NDV in titer of air (e.l.d.$_{50}$) corrected for efficiency of sampling (E = 95 %).

Intratracheal: The quantity of virus to infect 13 to 48 day old birds ranged from 50 to 100 ELD_{50}. The authors suggested that this evidence, in conjunction with respiratory results, was consistant with the hypothesis that the natural site of infection is the lower respiratory passage.

Intramuscular: The infections dose was in the order of 70 ELD_{50}.

Alimentary: Alimentary infection was initiated by doses of 20,000 ELD_{50} in chickens of 34 days old. The authors concluded that this route appeared to be the least sensitive of these examined to the HP virus strain and that it was probably the least important in the natural spread of the disease.

Table 5 summarises the results described above.

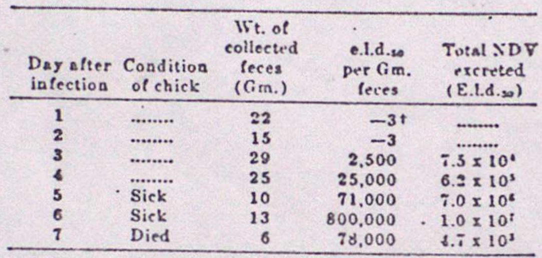
Table 5 - Comparison of infectivity of ND Virus administered to chicks by various routes.

Experiment No.	Age of chicken (days)	c.i.d.$_{50}$/(c.l.d.$_{50}$)† by route				
		Intranasal	Intratracheal	Respiratory (aerosol)	Alimentary	Intramuscular
1	70	5,000	......	—	....	.....
6	28	....	......	+50‡	....	.....
8	22	....	......	$\frac{110}{(250)}$	+800	.....
9	13	....	$\frac{51}{(+300)}$	—	....	.....
10	24	....	......	$\frac{133}{(435)}$	+3,000	.....
11	34	....	......	—	$\frac{20,000}{(63,000)}$	.....
12	48	....	100	—	....	.....
14	31	$\frac{4,000}{(4,500)}$	......	—	....	$\frac{45}{(400)}$
14a	8	....	......	—	....	30,000
17	7	25,000	250	—	....	370
Ave. c.i.d.$_{50}$ (experiments 1 to 14)		4,500	75	121	20,000	45

* In all experiments, with exception of No. 1, HP strain was used. In experiment 1, Komarov's Haifa vaccine virus was employed. † The numerators express the infections doses (50 %) for chickens in embryo lethal doses (c.l.d.$_{50}$). The denominators (in brackets) similarly express lethal doses for chickens. ‡ + = Titration end point not reached; more than the number stated.

Virus excretion in the faeces and from nasal mucous was also studied. After alimentary exposure to virus, virus was not detected in the faeces until the 3rd day and then it increased in magnitude until the 6th day see Table 6. The maximum virus recovered after intramuscular inoculation was recorded on the 5th day. A 25 mg faeces sample carried sufficient virus to infect a chick by the alimentary route.

Table 6 - Elimination of ND Virus in faeces of an infected chick*



Day after infection	Condition of chick	Wt. of collected feces (Gm.)	e.l.d.$_{50}$ per Gm. feces	Total NDV excreted (E.l.d.$_{50}$)
1	....	22	—3†	....
2	....	15	—3	....
3	....	29	2,500	7.5 x 10^4
4	....	25	25,000	6.2 x 10^5
5	Sick	10	71,000	7.0 x 10^6
6	Sick	13	800,000	1.0 x 10^7
7	Died	6	78,000	4.7 x 10^3

* Age of chick, 7 weeks. Infection with HP strain by intracrop instillation. † — = less than the number stated.

On one occasion the authors showed that a moribund chicken, with its own water supply, contaminated water with ND virus from nasal mucous; a 1 ml sample of the water contained 1000 ELD$_{50}$.

In conclusion the results indicated that the most probable port of entry of ND virus was the respiratory system, particularly the lower respiratory passages (trachea, bronchi and lungs). The relative sensitivity of chickens to infection by ND virus (HP strain) followed the sequence:- 1. intramuscular, 2. aerosol respiratory, 3. intratracheal, 4. intranasal and 5. alimentary.

Nadel and others (1957)

The infectivity obtained by inoculation of various quantities of ND Virus into embryonated eggs and 2 day old chicks were studied statistically to determine the probable minimum number of mature virus particles to inititiate infection. The observed embryo and chick mortalities were compared with theoretical mortalities based on two general hypothesis: 1. that the presence of at least r virus particles in the inoculum is sufficient to induce infection and 2. that the virus particles must attack at least r sensitive loci on the surface of the embryo cells or the chick brain cells before infection is accomplished. Poisson distributions based on assumed minimal numbers of viral particles needed to initiate infection were compared with the actual values derived from simultaneous inoculations of embryonated eggs and 2 day old chicks. The results, as presented in Tables 7 and 8 supports the conclusion that either one virus particle in the inoculum or a successful attack of one locus is sufficient to kill the embryonated egg. From two to five particles are needed to kill the 2 day old chicks. Under hypothesis 1. two particles in the innoculum are sufficient to kill the chick i.e. r = 2; but under 2. the number of sensitive loci which must be attached by the virus is probably two to four i.e. r = 2 to 4.

Table 7 - Numbers of live and dead embryonated eggs after indicated inoculations with ND virus, and comparison with the corresponding expected numbers obtained under various hypotheses regarding r.

Dilution (power of 10)	Observed		Expected numbers under given hypotheses					
			$r = 1$ (one-particle)		$r = 2$ (two-particle)		$r = 3$ (three-particle)	
	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
−7.8	24	0	22.0	2.0	50.4	3.6	52.2	1.8
−8.0	24	6	23.8	6.2				
−8.2	14	12	16.3	9.7	18.0	8.0	19.2	6.8
−8.4	10	17	12.6	14.4	12.0	15.0	11.8	15.2
−8.6	9	18	8.9	18.1	6.8	20.2	5.4	21.6
−8.8	5	20	5.5	19.5	2.9	22.1	1.9	23.1
−9.0	4	23	4.0	23.0	1.8	45.2	0.7	46.3
−9.2	0	10						
−9.4	0	10	1.6	18.4				
Chi-square			5.86		10.67		30.64	
Degrees of freedom			6		4		4	
P			0.44		0.03		<0.001	
Decision about hypothesis			Accept		Reject		Reject decisively	

Table 8 - Dilution-mortality relationships when 2-day-old chicks were inoculated with ND virus.

Dilution, X_i (power of 10)	Observed		$r=1$ (one particle)		$r=2$ (two-particle)		$r=3$ (three particle)	
	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
−5.3	36	0	77.4	0.6	114.8	3.2	116.4	1.6
−5.9	42	0						
−6.3	37	3	32.8	7.2				
−6.6	25	16	23.7	17.3	25.2	15.8	32.2	8.8
−6.9	8	30	13.3	24.7	11.2	26.8	12.9	25.1
−7.3	2	37	6.1	32.9	2.6	36.4	1.3	78.7
−7.6	0	41	3.5	37.5	0.8	40.2		
Chi-square			14.10		2.13		11.94	
Degrees of freedom			4		4		2	
P			0.009		0.57		0.006	
Decision about hypothesis			Reject		Accept		Reject	

Sinha and others (1957)

The severity of ND was studied in chickens acclimatised to different temperatures. The mortality in 6-7 week old birds, infected by several exposures with ND virus strain GB, was 100% at moderately high environmental temperatures (85 to 90°F), 95% at temperatures optimal for 5-6 week old birds (70 to 75°F), 75% at moderately low temperatures (50 to 55°F) and only 55% at low environmental temperatures 32 to 35°F). These results are shown in Figure 2. Figure 3 shows the course of disease in birds infected with virus excreted from the first batch. Tables 9, 10, 11 and 12 give the inculation periods associated with the experiments above.

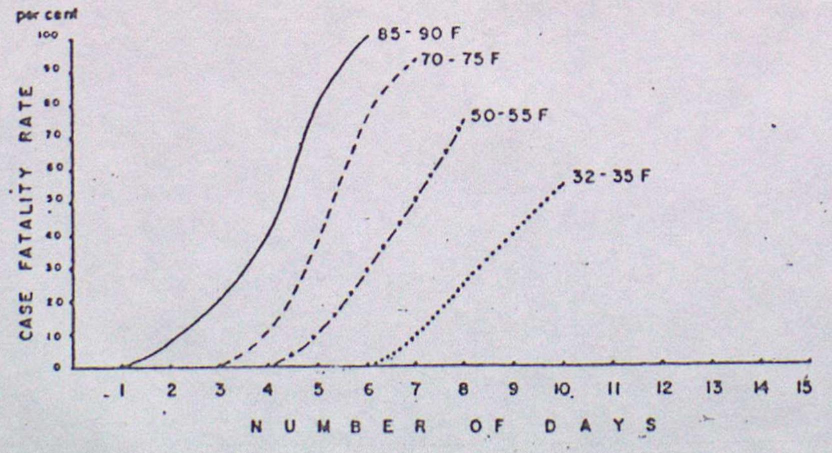


Fig 2 - Course of the diseased chickens following aerosol exposure with ND virus strai GB.

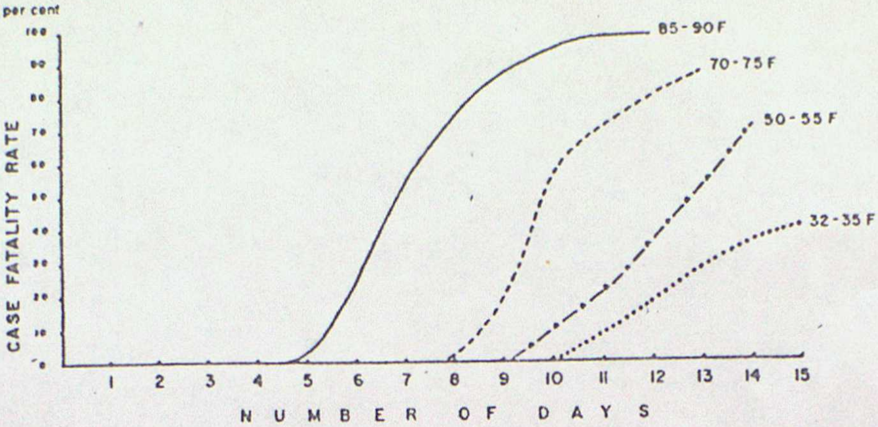


Fig 3 - Course of the susceptible birds infected by aerosol transmission from ND virus-infected chickens.

Tables 9-12 - Effect of environmental temperature upon severity of ND among chickens and on facility of aerosol transmission of the disease from infected to susceptible chickens.

Group A chickens exposed with ND virus (GB strain) by artificial aerosols.

Group B chickens infected by air-borne virus.

Table 9 - Environment temperature

85 - 90°F

Number of trials	Number of birds in groups	Average body temperature after 24 hr of acclimation	Incubation period in days	Number died	Number survived
Trial 1	10 Group A	107.6 F	3 to 4	10	0
	20 Group B	107.5 F	6 to 7	19	1
Trial 2	10 Group A	107.3 F	3 to 4	10	0
	20 Group B	107.4 F	6 to 7	20	0

Table 10 - Environment temperature

70 - 75°F

Number of trials	Number of birds in groups	Average body temperature after 24 hr in acclimation	Incubation period in days	Number died	Number survived
Trial 1	10 Group A	106.31 F	3 to 5	9	1
	20 Group B	106.4 F	6 to 8	17	3
Trial 2	10 Group A	106.55 F	4 to 5	10	0
	20 Group B	106.2 F	7 to 8	18	2

Table 11 - Environment temperature

50 - 55°F

Number of trials	Number of birds in groups	Average body temperature after 24 hr. of acclimation	Incubation period in days	Number died	Number survived
Trial 1	10 Group A	105.95 F	4 to 5	8	2
	20 Group B	106.05 F	8 to 9	13	7
Trial 2	10 Group A	106.15 F	4 to 5	7	3
	20 Group B	106.20 F	7 to 9	15	5

Table 12 - Environment temperature

32 - 35°F

Number of trials	Number of birds in groups	Average body temperature after 24 hr. of acclimation	Incubation period in days	Number died	Number survived
Trial 1	10 Group A	105.7 F	5 to 6	5	5
	20 Group B	105.9 F	9 to 10	7	13
Trial 2	10 Group A	106.0 F	5 to 7	6	4
	20 Group B	105.8 F	8 to 10	9	11

Asdell and Hanson (1960)

Asdell found that ND virus when introduced into 7-12 week old birds by inoculation, rapidly spread from the site of inoculation and could be detected in almost all tissues from 14 sites in the body within 48 hours and in all the tissues within 72 hours. Figures 4 and 5 show for two virus strains the recovered virus amounts from five parts of the chicken as a function of time.

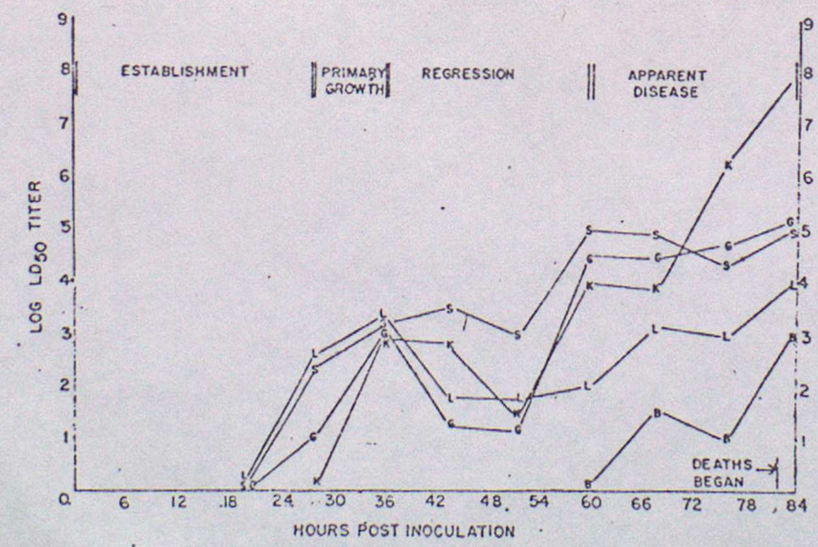


Fig 4 - Titre of the Texas-GB-1948 strain of ND virus in tissues of infected chickens. B = brain, G = lung, K = kidney, L = liver. S = spleen.

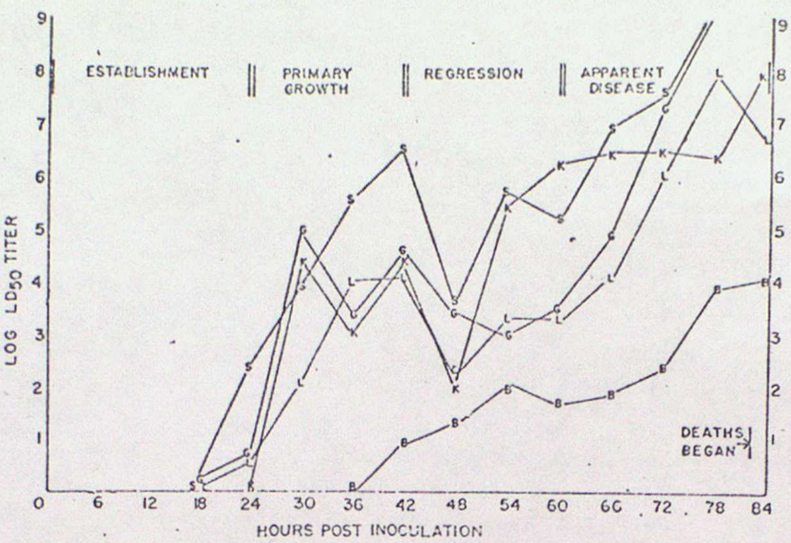


Fig 5 - Titre of the Italy-Milano-1945 strain of ND virus in tissues of infected chickens. B = brain, G = lung, K = kidney, L = liver, S = Spleen.

Andrews and Allison (1961)

Healthy chicks were exposed, for periods in excess of 24 hours, to chicks infected with the Boney strain of ND virus via the conjunctiva and nostril. The artificially infected birds were separated from the normal chicks by various distances. Table 13 indicates the number of chicks that died or survived in the experiment.

Table 13 - Infection of chicks across a barrier

+ and - signs indicated numbers of chicks dying (+) or surviving (-).

Barrier ...	+	−
Single wire	51	27
Double wire (2 in. separation)	12	1
Double wire (6 in. separation)	2	6
Double wire (9 in. separation)	0	10
Double wire (12 in. separation)	0	4
Metal 5 in. high	0	12
Metal 10 in. high	2	7
Glass 5 in. high	10	6
Glass 8 in. high	0	15

The authors also concluded that chicks were most infectious late in the course of the disease, particularly in the last 36 hours. Further experiments were performed to determine if increased exposure time of susceptible chicks to infected chicks and also increased numbers of infected chicks led to a greater chance of infection. The results show that with an exposure time of 24 to 48 hours there was a high risk of cross infection, whatever the number of birds initially affected and a much lower risk for a reduced exposure time.

An experiment was conducted to determine if infection occurred through the occular route in preference to the nasal route. Some of a group of chicks were given protective spectacles and all were exposed to airborne virus. The results indicated that infection via the nasal route was much more important than through the occular route.

Additional information with regard to airborne virus excretion was obtained when petri dishes were exposed at 10 and 25 cms from affected birds in an attempt to collect excreted virus. The results are shown in Tables 14 and 15.

Table 14 - Plaques from plates exposed in closed Topley Cages, housing infected chicks.

Time of exposure	Duration of exposure (min.)	Distance from carrier	
		10 cm.	25 cm.
2nd day after infection	60	0, 0, 0	0, 0
	120	0, 6, 0	0, 0, 0
3rd day after infection	30	22, 0, 0	0, 0, 0
	60	14, 6, 0	0, 0, 2
	240	8, 20	0, 0
Subterminal	30	18, 26	0, 0

Numbers separated by commas represent plaque counts in replicate plates.

Table 15 - Plaques from plates exposed in closed Topley Cages and open tanks, housing infected chicks.

Time of exposure	Duration of exposure (min.)	Closed Topley Distance from carrier		Open tank Distance from carrier	
		10 cm.	25 cm.	10 cm.	25 cm.
3rd day, Expt. 6	60	8, 5, 0, 0	0, 4, 0	0, 2, 0, 0	0, 0, 0
3rd day, Expt. 7	60	14, 6, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0

Hopkins and Dury (1971)

The authors assumed that some of the virus excreted became airborne and that by placing various grades of filters between infected birds (GB virus) and susceptible birds infection could be eliminated. Three types of filter were used:

roughing = 10 to 60% removal, medium-efficiency = 60 to 90% removal and high-efficiency = 90 to 99% removal for particles in the size range of 1 to 5 um. The results indicated that there was a 6%, 34% and 74% reduction in infection for air filtered with the roughing, medium efficiency and high-efficiency filters respectively, compared with a no filter control sample.

The evidence presented gives weight to the argument that some excretion becomes airborne and is associated with particles of a few micross in size.

Hugh-Jones et al (1973)

This paper describes measurements taken both in the laboratory and in the Field. Three strains of virus were used; Herts' 33/56, Eastwood '67 and Essex '70. For convenience the work has been divided into 3 sections.

Excretion of virus (Laboratory)

Five 6 week old birds were inoculated with Herts '33/56 and then they were placed in contact with 15 other birds. The relative humidity during the experiment ranged from 30-60%. 60 minute air samples were taken throughout the period using a Litton sampler. Analysis of the fluid from the Litton sampler (equivalent to 200L of air) showed that virus was present at least 12 hours before the death of the inoculated birds. The 15 in contact birds started to die about 100 hours after release of virus from the donor birds. Figure 6 shows the bird mortality and virus excretion throughout the period.

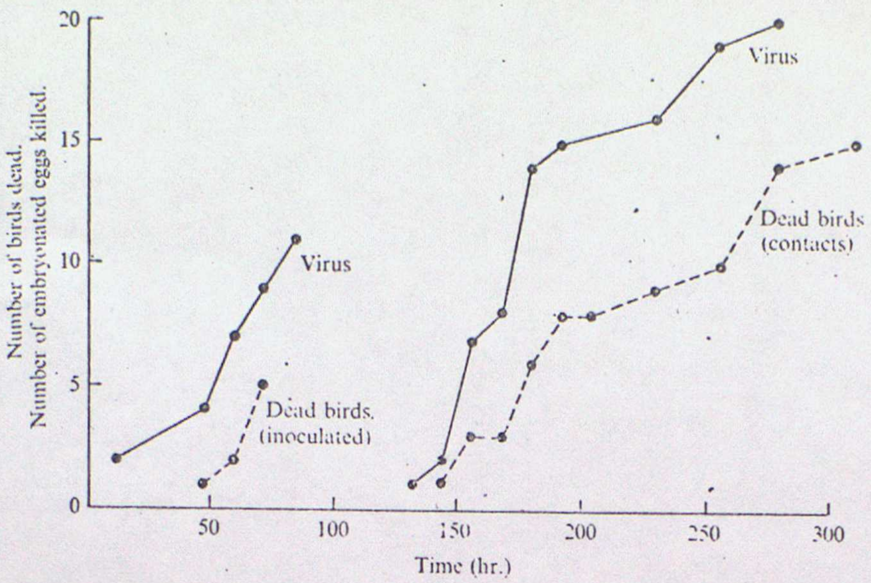


Figure 6 - Bird mortality and airborne virus recovered from contact experiment. (Virus concentration is expressed as the cumulative number of embryonated eggs killed when inoculated with 0.1 ml. (≙200.L air) of fluid from the Litton Air Sampler).

The experiment was repeated using Essex '70 field strain virus. The results differed from the previous experiment in that the birds were killed more rapidly (all 15 in-contact birds were dead by 238 hours) and that virus concentrations in the air were higher (at 188 hours a 0.1 ml sample from the Litton contained 280 ELD_{50}).

Survival - the effect of changing the relative humidity (Laboratory)

Aerosols of all three strains were generated using a Collison atomiser and then stored in the dark at a variety of relative humidities. Table 16 gives the results although these are somewhat confusing. It is probably safe to draw the conclusion that some virus remains viable after 4 hours.

Virus strain	R.H.	30 min.	60 min.	240 min.
Essex '70	80	36	12	6
	70	49	95	15
	60	40	33	9
	50	13	7	2
Herts '33/56	80	96	53	11
	80	67	—	11
	70	88	47	7
	60	18	14	3
	50	21	23	3
Eastwood '67	80	40	17	5
	70	64	38	10
	60	111	52	9
	60	34	39	4
	50	2	1	1

Table 11 - Viability of three strains of ND Virus after holding in the rotating drum at 20°C for various times and at different relative humidities (RH).

In a further set of experiments deoplets containing virus were suspended on a ultra fine spiders web. These webs were then exposed to a range of humidities and air from different outside sources. Significant quantities of virus survived for at least 30 minutes. The results are shown in Table 17 but once again are not clear cut.

Table 17 - The viability of three strains of ND Virus held in the open air at various relative humidities (RH) and in winds from various directions. (The control at 0 min was given the nominal value of 100%. The viability of E coli 162 (EC) exposed at the same time is given for comparison).

		Wind			Time of exposure		
Strain	R.H.	Direction	Speed (m./sec.)	Organism	30 min.	60 min.	Control, 60 min.
Essex '70	60	040°	25·5	NDV	62	31	36
				EC	26	8	79
	63	340°	17	NDV	24	8	4
				EC	10	3	84
	53	250°	20·4	NDV	20	0	18
				EC	9	3	41
	62	245°	17	NDV	22	5	28
				EC	6	3	74
	93	120°	8·5	NDV	11	0	46
				EC	5	0	82
Herts '33/56	75	290°	17	NDV	23	23	36
				EC	29	42	39
	95	190°	46	NDV	48	7	6
				EC	43	21	93
	70	309°	36	NDV	7	11	52
				EC	34	8	63
	78	070°	8·8	NDV	5	0	35
				EC	1	2	70
Eastwood '67	63	050°	18·8	NDV	30	—	38
				EC	3	—	76
	60	270°	18·8	NDV	212	59	189
				EC	20	2	103
	65	220°	24	NDV	1	< 1	4
				EC	28	13	81
	65	270°	27·2	NDV	51	3	9
				EC	36	6	130
	68	070°	13·6	NDV	5	0	35
				EC	6	1	68

Recovery of virus from infected premises (field)

Three farms at which there were reported outbreaks of ND were used. The first farm consisted of 70,000, 21-23 day old birds kept in eight houses. The second farm had 43,500, 38 day old chickens housed in 4 units and the third 12,750, 37 day old birds chickens.

Air samples were taken at the first and second farms (1000 l/min into 28 ml of fluid for 30 minutes) at various locations inside and out of the houses. Table 18 gives these results. At the third farm a size distribution of the particles carrying ND virus was taken. The results are shown in Table 19.

Table 18 - Amount of virus recovered from samples taken at various positions in and around two farms where there were outbreaks of Newcastle disease.

Sample position	Site no.*	R.H.	Temp. (°C.)	Virus recovered†
Farm A. (Samples taken at night, wind speed 0·4–0·7 m./sec., 280–295°; 8/8 cloud.)				
Upwind (21 m.)	1	94	6·1–6·9	Trace‡
Inside house	4	67	21·0–21·8	5·7
Downwind (23 m.)	3	96	4·2–4·4	2·95
Downwind (55 m.)	2	92	5·3–5·5	2·0
Farm B. (Samples taken in daylight, wind speed 2·9–3·9 m./sec., 130–145°; 6/8–8/8 cloud)				
Upwind (101 m.)	1	82	8·3	Trace‡
Inside house	4	70	15–15·5	5·28
Downwind (64 m.)	3	79	8·3–8·6	1·45
Downwind (165 m.)	2	84	7·8–8·3	Nil

* See Fig. 1.

† Virus titre, $\log_{10}$ ELD 50 per 33,000 l. of air.

‡ Trace = too low for accurate estimation.

Table 19 - The size distribution of the particles carrying Newcastle disease virus recovered from air inside a poultry house at farm C.

Particle size (μm.)	Stage of multistage sampler	Virus recovered* At litter level	Virus recovered* 1·7 m. above floor
> 6	Top	3·39	3·11
3–6	Middle	3·14	3·31
< 3	Bottom	1·68	1·97

* Virus titre = $\log_{10}$ ELD 50 per 1000 l. of air.

Comparison of virus from vaccinated and unvaccinated birds

Batches of 90, 56 day old birds were split into two groups. Half of the birds had been vaccinated with La Sota vaccine at day 21 and 35. They were all challanged with $4 \times 10^{10.6}$ ELD_{50} Essex '70 in an aerosol form. From the 4th to 6th day after challange the air was sampled for 30 minutes using a multistage liquid impinger. The results are shown in Table 20 and it is obvious that a much larger amount of virus is excreted by the non vaccinated birds.

Table 20 - Comparison of the amounts of ND Virus recovered from vaccinated and non-vaccinated birds.

No. of days after challenge		Birds Live	Birds Dead	Virus recovered*
4	Vaccinated	90	0	2·72
	Non-vaccinated	86	4	4·66
5	Vaccinated	90	0	2·24
	Non-vaccinated	47	43	4·18
6	Vaccinated	90	0	2·20
	Non-vaccinated	23	67	2·87

* Virus titre, $\log_{10}$ ELD 50 per 1000 l. air.

Estola and others (1979)

As part of an experiment to determine whether airborne disease spread could be eliminated by using air ionization, 1-5 week old birds were inoculated intracheally with a velogenic strain of ND virus ($0.3 \times 10^{8.7} ELD_{50}$). These were housed with indicator birds. The experiments were performed with and without air ionization. See Table 21.

Table 21 - Inhibition by air ionization of air-borne transmission of ND virus in chicken groups

Ion generator	Chicken group	Deaths per consecutive day after inoculation Day... 2 4 6 8 10 12 14 16 18 20	Total
	Experiment I		
	(A) Inoculated	– – 8	8/8
Off	(B) Indicator	– – – – – 1 – 1 1 1 – 2	6/8
	Experiment II		
On	(A) Inoculated	– – 1 3 – 2	6/6
	(B) Indicator	– – – – – – – – – – – – – – – – – – – –	0/6

Spreading of the virus from group A to B was scored by recording the date of death of the indicator chickens. After the first experiment under normal conditions (I) the room and cages were decontaminated, and a similar transmission experiment was performed with the ion-generating apparatus on. (–, No deaths recorded).

The results indicate that ionised air reduces the chance of airborne disease spread.

Virus Survival

Farinas (1930)

The author found that virus was inactivated in 30 minutes at 55°C but remained infectious at 50°C. At 37°C it was active after 24 hours but not after 72 hours.

Virus infected saliva was diluted and exposed to morning and afternoon sunlight in the Philippines. After a period of 30 minutes the virus became inactivated.

Doyle (1933)

ND virus remained viable in bone marrow for at least 6 months held under "trade chilling" conditions.

Iyer (1943)

ND virus was killed by heating it above 50°C. Virus suspensions exposed to direct sunlight for 1 hour remained infective.

Levinson (1944)

ND virus was exposed to ultra-violet rays (1600 to 1800 Å) and for the strains used inactivity occurred in 0.8 to 1.08 seconds.

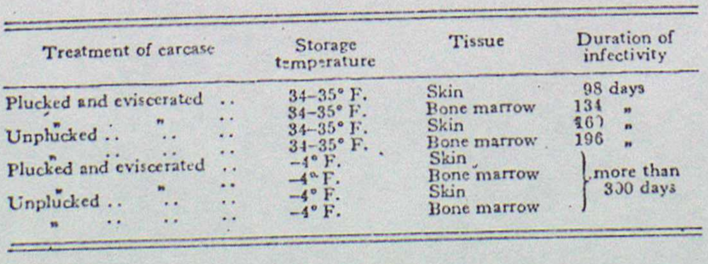
Moses (1947)

Infectivity of ND virus was retained for many hours in the PH range 2 to 10.

Asplin (1949)

Asplin examined the viability of Herts '33. Eight cockerels were inoculated with virus and when symptoms occurred (4 days later) the fowls were killed and stored under a variety of conditions. The results are shown in Table 22. In addition the survival of virus under several different laboratory conditions was determined. The results are given in Table 23.

Table 22 - Survival of ND virus in stored infected carcase.



Treatment of carcase	Storage temperature	Tissue	Duration of infectivity
Plucked and eviscerated ..	34–35° F.	Skin	98 days
" ..	34–35° F.	Bone marrow	134 "
Unplucked	34–35° F.	Skin	160 "
"	34–35° F.	Bone marrow	196 "
Plucked and eviscerated ..	–4° F.	Skin	more than 300 days
" ..	–4° F.	Bone marrow	
Unplucked	–4° F.	Skin	
"	–4° F.	Bone marrow	

Table 23 - Survival of ND virus under laboratory conditions

Treatment of virus	Result
Exposed on filter paper to dry heat at 98° F.	Active after 6 hours. Inactive after 12 hours
Exposed on egg shell to dry heat at 98° F.	Active after 24 hours. Inactive after 44 hours
Exposed on hessian in sterile faeces to dry heat at 98° F.	Active after 72 hours. Inactive after 96 hours
Fluid virus suspension in water bath at 133° F. (56° C.)	Active after 45 min. Inactive after 60 min.
Exposed on filter paper stored at :— (*a*) 34°–35° F. .. .. .. (*b*) 32° F.	Active after 203 days. Inactive after 217 days " 161 " " 175 "
Dried on glass, stored at :— (*a*) 34°–35° F. ; (*b*) 32° F. ..	Still active after 396 days

Foster and Thompson (1957)

These authors concluded that ND virus is destroyed in 1 minute at 100°C, 5-6 minutes at 56°C, hours or days at 37°C, months and years at 20°C and 8°C.

Bushnell and Erwin (1950)

The authors investigated several strains of ND virus in the form of allanto-amniotic fluids and concluded that the thermal death point of the virus was between 58°C and 64°C for a 30 minute exposure.

Olesuik (1951)

The results of the experiments performed by Olesiuk are given in Table 24. These show the effects of exposure time and storage temperature for ND virus on different substrates.

Table 24 - Duration of viability of ND virus.

	Incubator 37 C. (Days)		Normal room 20 to 30 C. (Days)		Hen house −11 to 36 C. (Days)	Refrigerator 3 to 6 C. (Days)	Deep freeze −26 C. (Days)
Test material	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 1	Trial 1
Burlap	*	14	16	9	108	123	538**
Filter paper	*	28	25	49	129	157	538
Cloth	10	16	52	35	145	193	538
Broth	46	14	192	112	228	538	538
Saline	17	35	87	71	228	451	538
Eggshells	*	7	52	35	228	258	538
Mash	52	56	66	56	172	258	538
Chicken feces	25	56	94	71	172	538	538
Down	87	28	192	134	255	538	538
Soil	25	2	60	71	172	235	538
Eggs	94	126	235	197	255	538	...
A.A.F.***	31	35	66	94	199	258	538

*The end point of viability was less than ten days. **The duration of the study was 538 days. ***Undiluted amnioallantoic fluid.

Skinner and Bradish (1954)

The authors found that by exposing solutions of ND virus for 4 hours at 4°C to natural light that there was a decrease of titre of 2.7 and 1.2 when the ambient light intensities were 2600 Lux and 2150 Lux. For samples, held at 4°C for 4 hours under artificial light (Mercury vapour) there was $\geqslant$ 3.4 decrease of titre at 41,000 Lux, 1.3 titres at 6460 Lux and 0.6 titres at 410 Lux.

They concluded that their evidence was not inconsistent with the hypothesis that the decrease of infectivity of a virus suspension by exposure to light could be determined by the product of the light intensity and the duration of exposure.

Boyd and Hanson (1958)

Investigations were made into the survival of GB Texas strain virus stored on paper discs at three relative humidities (0, 15 and 100%). The results are shown in Table 25 and Figure 7. The experiment was repeated at 25°C and 100% relative humidity see Table 26.

Table 25 - Survival of ND virus, 10^{10} ELD_{50}, in paper discs and soils at 20 ± 2C.

	100% Relative humidity			9·15% (Room) Relative humidity			0% Relative humidity		
Time (days)	Paper discs	Soil Sterile	Soil Non-sterile	Paper discs	Soil Sterile	Soil Non-sterile	Paper discs	Soil Sterile	Soil Non-sterile
0*	10.0**	9.5	9.5	10.0	9.5	9.5	10.0	9.5	9.5
1	9.5	—***	—	7.8	—	—	6.6	—	—
2	—	—	—	—	—	—	6.2	—	—
3	8.3	—	—	6.5	—	—	>5.5	—	—
4	—	8.3	7.4	—	7.6	6.8	—	7.6	6.0
5	7.9	—	—	5.4	—	—	—	—	—
6	7.6	—	—	4.6	—	—	>6.5	—	—
8	—	7.8	8.3	—	7.3	7.5	—	7.4	6.4
9	6.2	—	—	>5.5	—	—	4.5	—	—
15	—	5.3	5.3	—	<2.0	<2.0	—	<2.0	<2.0
17	4.0	—	—	<2.6	—	—	4.6	—	—
20	2.8	—	—	2.8	—	—	3.0	—	—
22	—	3.3	3.3	—	—	—	—	—	—

*Titration at 1 hour

** Log ELD_{50} of conc of virus

*** No recovery attempted.

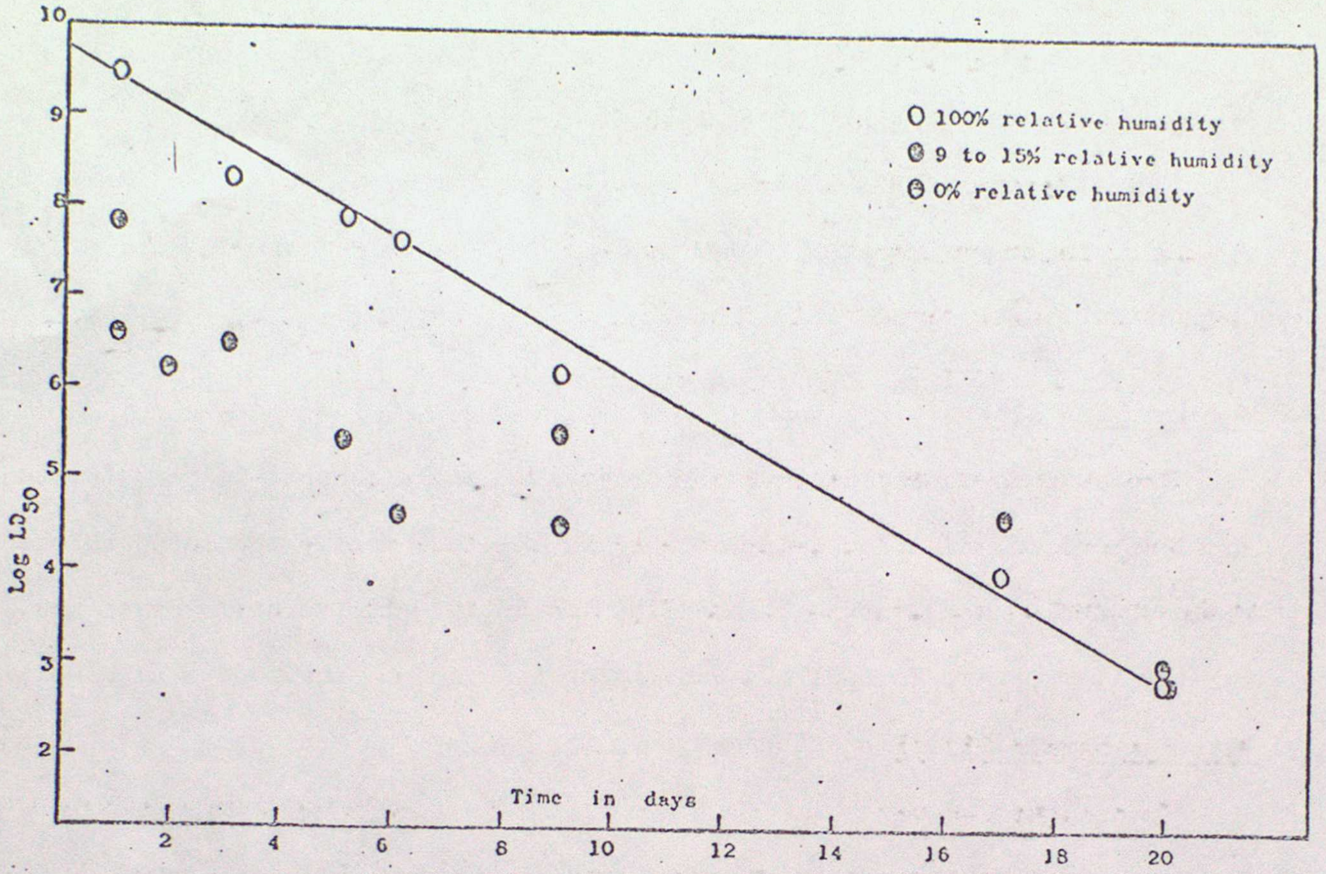


Figure 7 - The survival of ND virus in paper discs at 20±2C and at three relative humidities

Table 26 - Survival of ND virus, 10^{10} ELD_{50}, in soil at 25 C and 100 percent relative humidity.

Time	Sterile	Non-sterile	Sterile + bacteria
1 hour	9.5*	9.5	9.5
24 hours	8.6	8.2	8.5
72 hours	8.5 (8.0)**	7.4 (6.7)	7.6 (8.0)
5 days	6.5	6.5	7.4
6 days	5.6	5.6	6.0

* Log ELD_{50} conc of virus
** Replicate

AIRBORNE TRANSMISSION OF DISEASE (FIELD)

Jungherr and others (1946)

The authors concluded that inclement weather during the winter months may have acted as a modifying or predisposing factor in ND outbreaks which occurred in 1945 and 1946 in Connecticut, USA.

Moynihan and others (1951)

The authors concluded that airborne virus could account in part for the rapid spread of infection, particularly in the more densly populated chicken regions, during a ND outbreak in British Columbia. The virus involved was relatively avirulent for pullets and laying hens and produced a high morbidity but a low mortality. In outbreaks involving younger birds there was a high mortality

Mansjoer (1961)

A virulent epozoatic of ND in Bandung and Bogor, Indonesia occurred during the 1960/1961 dry season. The authors believed that it was the susceptability of the poultry population and the other opportunities for dissemination of the virus rather than the weather that influenced disease spread. Other workers in Indonesia had reported that ND was more prevelant in the wet and changing season. In addition to these findings the authors reported that they had isolated virus from the air from infected poultry houses in Tjiandjur during 1948 and 1955.

Drummond and Hunnam (1961)

Three separate outbreaks of ND in East Sussex during 1959/60 were examined; all of the outbreaks were caused by a mesogenic form of ND virus. Windborne transmission of the disease was believed to be the cause for the substantial part of disease spread. The outbreaks tended to be separated by distances of over a half a mile and on occasions by several miles. One of these outbreaks is described in detail by Gloster (1982b).

Smith (1964)

Two outbreaks of ND in Bedfordshire and neighbouring counties during 1960 and 1962 were studied to determine if meteorological conditions influenced ND spread. The results based on meteorological evidence alone indicated that windborne transport of virus could account for much of the secondary infection. Distances up to 35 km could easily have been involved.

Gilmore (1971)

The author described an outbreak of ND in Kent during 1969. No explanation for disease spread could be found other than by the airborne route. This outbreak has been investigated in detail by Gloster (1982a).

Anon

Dr Wilsden, a retired MAFF veterinary office, made available a copy of a report that he had in his personal possession. This article had been written about the factors which influence the spread of ND. It has been impossible to trace the exact origins of this document.

One section concludes with the statement that local spread of ND occurs downwind of large broiler units. Several examples are given in the article including the one given in Table 27. This table gives a breakdown of disease and disease origins for outbreaks in East Sussex during 1960 (January to April). The author concluded that infection from several broiler units was responsible for the majority of secondary outbreaks.

The author also found that outbreaks finished more quickly in the Spring and Summer months rather than during the Autumn and Winter months.

SUMMARY

The literature search has shown that infected birds excrete virus into the atmosphere, particularly from the respiratory tract. This excretion starts several days prior to the appearance of visible clinical symptons and reaches its maximum when they are most apparent.

Table 27 - Disease and disease origins in East Sussex during 1960 (January to April).

1960	East Sussex		
Month	No. and Classification of Outbreaks		Origin
	Primary	Secondary	
January	1		Swill
	1		Visit of Poultry Slaughterer
	2		Obscure
		3	Due to local spread
		60	Due to local spread from a 1959 broiler outbreak
February		46	Due to local spread from a 1959 broiler outbreak
March		1	Sale from Hants.
		1	Personal contact
		8	Due to local spread
April		2	Due to local spread
	4	121	Total 125

Measurements, taken from the air inside and outside of infected poultry houses, indicate that there are a significant number of particles containing virus. Many of these particles are less than ten microns in size.

The survival of the virus depends upon the temperature, relative humidity, light levels and pH. At deep freeze temperatures the virus remains viable for years, at normal room temperatures up to several hundred days, at 40°C up to a hundred days and at greater than 50°C only minutes. In all the experiments with varying relative humidities the virus survival appears to be inversely related to humidity; the minimum viability was of the order of hours. The most detailed experiments on the effect of light intensity and exposure time suggest that virus survival is related to the product of light intensity and duration of exposure. With the intensity of light often found in these climes the duration of exposure would need to be many hours to appreciable effect viability. Infectivity of ND virus was retained for many hours in the range 2-10 pH.

Infection can be initiated via the respiratory alimentary and occular route or directly by inoculation into the muscle. The natural port of entry appears to be the respiratory system, particularly the lower respiratory passage. The dosages required to infect birds appears to be low and young birds are most susceptible. After infection there is a period of several days before the virus is excreted in significant quantities from the respiratory system and in the faeces.

Evidence was presented which indicated that airborne transmission of disease has occurred in the field.

Some areas requiring further research

The literature review has revealed certain short-comings in our knowledge on the transmission of ND. These short-comings need to be eliminated before an accurate assessment of disease spread by the airborne route can be made.

1. A more quantitative estimate is required of the number of virus particles excreted as a function of:

    Time
    Virus strain (virulent to avirulent)
    Age of bird

2. The composition of particles containing virus, selected in the atmosphere, needs examination. What part do atmospheric nuclei, feather follicles, dried excreta etc play in determing the airborne concentration of virus?

3. Natural infection has been shown to occur predominately via the respiratory system. Additional information is required to determine the minimum number of particles required to cause infection by this route. The effect of bird age upon susceptibility to airborne infection requires study.

4. Confirmation of the viability of airborne particles containing virus is required. Particular attention needs to be placed on the time scale of a few hours at a range of ambient light levels and values of relative humidity.

5. Detailed studies of past disease outbreaks are required to confirm that airborne transmission of disease occurs in the field.

6. The effect of chicken population size and the likelihood of infection requires examination. For a given inhaled dosage would a unit of 100,000 birds be at a greater risk than one of 100 birds?

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